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## ANOPHELES PUNCTIPENNIS SAY.

### ITS RELATION TO THE TRANSMISSION OF MALARIA—REPORT OF EXPERIMENTAL DATA RELATIVE TO SUBTERTIAN<sup>1</sup> MALARIAL FEVER.

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The wide occurrence of *Anopheles punctipennis* Say, in the United States and its biologic relations in certain localities where malarial incidence has been investigated by the United States Public Health Service, have made it highly desirable more closely to investigate the possible rôle played by this insect in the transmission of malarial fevers.

The only record of previous work, available in literature, is that of Hirshberg.<sup>2</sup> This, while complete in itself, was not considered by Surg. R. H. von Ezdorf, in charge of malarial investigations, to be adequate definitely to exclude this anopheline as a carrier. It was under Dr. von Ezdorf's direction that the present researches were undertaken.

It is here presumed that negative results can not, as is well known, be concluded unless a large number of specimens have been used under the most favorable conditions. On this account an effort was made, in the experiments outlined, to make the study as exhaustive as the material would permit. It is not, however, advanced that the results obtained from experimental procedure furnish a true criterion of what occurs under natural environments. In the present experiments no special attempts were made to simulate natural conditions; indeed, a relatively low temperature was sustained throughout, in order to insure longevity of the insects under observation, and the majority of the parasitized mosquitoes were kept for long periods prior to dissection. In addition, the number of feedings was made sufficient to secure the maximum possibilities for infection.

The present investigation was limited to subtertian malaria, as suitable material for similar studies with the tertian variety, which had also been planned, was not available at the inception of the work.

Two series of experiments were attempted; one, with recently emerged mosquitoes and a heavily infected carrier undergoing quinine treatment; the other, with older mosquitoes and a lighter human infection, untreated. The anophelines used were bred mosquitoes, which emerged August 25–26, 1915. The material for the second series was kept for 29 to 30 days prior to any experimental use by feeding on a healthy laboratory attendant.

The containers employed were lantern chimneys kept in deep glass trays, lined with a layer of absorbent cotton, covered with

<sup>1</sup> Subtertian is here used synonymously with estivo-autumnal.

<sup>2</sup> Hirshberg, L. K. An *Anopheles* mosquito which does not transmit malaria. Bull. Johns Hopkins Hospital, Baltimore. Vol. XV, No. 155, Feb., 1904, pp. 53–56.

heavy blotting paper and kept constantly saturated with water. Food material of raisins and prunes was placed from time to time on the upper ends of the screened chimneys. The temperature maintained throughout the series was 21 to 22° C. in an electric low temperature incubator. Here there was a fluctuation of  $\frac{1}{2}$  to 1 degree by the use of ice in the cooling chamber of the incubator. In the second series ice was not employed, the temperature thus influenced by that of the room having a greater range; it registered 18 to 24° C.

The material available for the initial infection of the experimental mosquitoes is stated in the following table:

TABLE No. 1.—*Giving the number of mosquitoes applied daily and percentage of gametocytes present in the blood.*

Date.	Gametocyte count <sup>1</sup> per 100 leucocytes.	Number of <i>Anoph. punctipennis</i> observed to bite.	Number of <i>Anoph. quadrimaculatus</i> observed to bite.	Date.	Gametocyte count <sup>1</sup> per 100 leucocytes.	Number of <i>Anoph. punctipennis</i> observed to bite.	Number of <i>Anoph. quadrimaculatus</i> observed to bite.
1915.				1915.			
Sept. 4.....	16	7	5	Sept. 19.....	29	63	.....
7.....	33	15	.....	20.....	15	49	.....
9.....	16	27	.....	21.....	3	31	.....
10.....	48	23	8	22.....	4	32	.....
11.....	69	23	4	23.....	5	21	.....
12.....	33	37	2	24.....	3	39	.....
13.....	30	7	6	25.....	2	21	.....
14.....	28	50	3	26.....	2	8	.....
15.....	24	44	2	27.....	3	10	.....
16.....	17	38	.....	Total.....	664	30	.....
17.....	9	57	.....				
18.....	18	62	.....				

<sup>1</sup> Determinations made from thick and thin blood smears, stained, counting 200 to 400 leucocytes.

Table No. 1 gives the number of bites received by 152 specimens of *A. punctipennis*, which survived three days or longer and were suitable for dissection; the others are not tabulated. It will be seen that during the period September 4 to 27 the patient was bitten 664 times. The mosquitoes were applied twice daily and were permitted to engorge themselves. The specimens of *A. quadrimaculatus* used as controls in this experiment were observed to bite 30 times.

In order to gauge accurately the proportion of mosquitoes in which bloodsucking occurred, the insects from the various cages are grouped in the following table:

TABLE NO. 2.—*Giving the average number of bites obtained by mosquitoes (*A. punctipennis*) grouped as to cages.*

Cage No.	Number mos-quitos applied.	Period of feed-ing. <sup>1</sup>	Num-ber of bites per group.	Aver-age num-ber of bites per mos-quito.	Cage No.	Number mos-quitos applied.	Period of feed-ing. <sup>1</sup>	Num-ber of bites per group.	Aver-age num-ber of bites per mos-quito.
1.....	4	3	6	1.5	12.....	14	14	81	5.7
2.....	7	5	21	3.0	13.....	4	14	38	9.5
3.....	7	3	14	2.0	14.....	13	14	83	6.4
4.....	4	5	8	2.0	15.....	8	14	45	5.6
5.....	4	3	19	4.7	16.....	8	14	52	6.5
6.....	3	3	14	4.6	17.....	15	14	68	4.5
7.....	7	7	18	2.5	18.....	12	14	58	4.8
8.....	4	5	20	5.0	19.....	16	9	32	2.0
9.....	3	11	20	6.6	20.....	6	9	14	2.3
10.....	2	11	12	6.0	21.....	8	9	32	4.0
11.....	3	11	9	3.0					

<sup>1</sup> Period of successive days during which mosquitoes were applied repeatedly.

#### SUMMARY.

Number of mosquitoes.	Number of bites.	Number of mosquitoes.	Number of bites.
4.....	1-2	22.....	5-6
27.....	2	2.....	6
13.....	2-3	24.....	6-7
10.....	3	4.....	9-10
8.....	4		
34.....	4-5	152 <sup>1</sup> .....	2 4.3
4.....	5		

<sup>1</sup> Total.

<sup>2</sup> Average.

It will be observed from Table No. 2 that the mosquitoes applied and later dissected received an average of more than four feedings, ranging from 1 to 10 bites per mosquito.

In the following table recording dissections, the counts are tabulated beginning after the initial feeding on the infected host. Only toward the end of the experiment were the mosquitoes killed for the purpose of examination; for the most part the dissections were made of feeble or dying specimens.

TABLE NO. 3.—*Tabulating the dissections of *A. punctipennis* beginning three days after biting.*

Number of days after biting.	Number of mosquitoes dissected.	Number of days after biting.	Number of mosquitoes dissected.
3.....	6	21.....	2
4.....	6	23.....	5
5.....	9	24.....	6
6.....	7	25.....	5
7.....	6	26.....	2
8.....	8	27.....	2
11.....	2	29.....	12
12.....	1	30.....	7
13.....	2	31.....	7
14.....	1	32.....	8
15.....	6	33.....	15
16.....	2	36.....	1
17.....	4	37.....	1
18.....	6	38.....	1
19.....	3		
20.....	9	Total.....	162

It is to be noted in connection with this series that the individual upon whom these mosquitoes were allowed to feed was at the time undergoing quinine medication. From September 6 to 9 he was given 120 grains of quinine bisulphate, and September 10 to 19, 400 grains, all in acid solution. He received no quinine from September 20 to 27, after which date observations were discontinued.

For the further determination of the possible infectivity of these *A. punctipennis*, two healthy persons who had never suffered an attack of malarial fever and whose blood was negative upon repeated examinations, volunteered to permit these mosquitoes to bite them. The mosquitoes were applied in two lots from 4 to 33 days after feeding upon the blood of the individual whose gametocytes are enumerated in Table No. 1. The table which follows presents the number of bites received by the two volunteers:

TABLE NO. 4.—*Detailing the control human experiments in attempting to transmit by A. punctipennis.*

Number of days elapsing since biting malaria carrier.	Number of mos-quites biting volunteers.		Number of days elapsing since biting malaria carrier.	Number of mos-quites biting volunteers.	
	M. B. M.	H. E. H.		M. B. M.	H. E. H.
4.....	7	.....	19.....	6	1
6.....	3	.....	20.....	1	6
7.....	.....	9	21.....	.....	4
8.....	10	3	22.....	2	5
9.....	3	8	23.....	2	11
10.....	11	7	24.....	1	.....
11.....	2	3	25.....	2	.....
12.....	12	12	26.....	.....	7
13.....	2	10	27.....	1	17
14.....	.....	25	28.....	3	1
15.....	9	17	33.....	.....	4
16.....	4	14	Total.....	91	180
17.....	10	10			
18.....	.....	6			

The experiments were controlled by the parallel feeding of 14 specimens of *A. quadrimaculatus* on the same infected individual used throughout series 1. These mosquitoes were reared from larvae in the laboratory and applied in two groups, averaging one to two bites for each specimen. Two of these showed infection; one, after an incubation of eight days, showed 12 sporoblastic oocysts of various sizes, one of which was mature; the second, after 17 days incubation, showed one large and several half-grown oocysts. The sporozoites of the ripe oocyst when stained appeared indistinguishable from similar bodies crushed from the salivary glands of infected mosquitoes; of these there were counted 205, which, with the exception of 43, were apparently mature.

An accidental check by the bite of the second infected mosquito, *A. quadrimaculatus*, occurred inadvertently in this experiment, one of the personnel of the laboratory force being bitten on September 28, 15 days after this insect had fed on the blood of the gametocyte

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carrier. A sharp attack of subtertian malarial fever followed after an incubation period of 11 days, the initial paroxysm occurring on October 8, and characteristic ring forms of the subtertian parasite being found in the peripheral blood; treatment was deferred for three days, during which the diagnosis was amply substantiated.

### Series 2.

In series 2, it was thought desirable to employ older specimens of *A. punctipennis* to determine if any age differences influenced the susceptibility of mosquitoes to plasmodial infection. In the second series, the mosquitoes used were at least 29 to 30 days old at the beginning of the experiment. One of these survived to the age of 82 days, and another lived 111 days.

The case was one of subtertian malarial fever which had not received any specific treatment during the 14 days prior to use in the infection experiments, specific treatment being withheld also during the six days mosquitoes were applied. Table No. 5 outlines the number of bites by the mosquitoes and the gametocyte counts during the six days, October 25 to 30, 1915.

TABLE NO. 5.—*Giving the record of biting of subtertian carrier by Anopheles punctipennis and controls.*

Date.	Gametoctye count <sup>1</sup> per 100 leucocytes.	Num-ber of <i>A. puncti-pennis</i> biting.	Num-ber of <i>A. quadri-maculatus</i> biting.	Num-ber of <i>A. crucians</i> biting.	Date.	Gametoctye count <sup>1</sup> per 100 leucocytes.	Num-ber of <i>A. puncti-pennis</i> biting.	Num-ber of <i>A. quadri-maculatus</i> biting.	Num-ber of <i>A. crucians</i> biting.
Oct. 25.....	20	37	21	3	Oct. 28.....	4	24	20	.....
26.....	7	34	18	1	29.....	3	19	22	.....
27.....	6	27	16	2	30.....	3	17	7	.....

<sup>1</sup> Determined in stained thick and thin blood smears; 200 to 400 leucocytes counted.

In this series, 67 specimens of *A. punctipennis*, 60 specimens of *A. quadrimaculatus*, and 3 specimens of *A. crucians* were dissected. These are tabulated in Table No. 6.

TABLE NO. 6.—*Showing number and species of mosquitoes dissected in series No. 2.*

Number of days elapsing since biting malaria carrier.	<i>Anopheles punctipennis.</i>	<i>Anopheles quadrimaculatus.</i>	<i>Anopheles crucians.</i>	Number of days elapsing since biting malaria carrier.	<i>Anopheles punctipennis.</i>	<i>Anopheles quadrimaculatus.</i>	<i>Anopheles crucians.</i>
4.....	2	6	.....	17.....	1	1	.....
6.....	6	6	.....	18.....	11	3	1
7.....	1	6	.....	19.....	13	1	.....
8.....	2	2	.....	20.....	5	2	.....
9.....	3	8	.....	21.....	5	2	.....
10.....	2	11	.....	22.....	3	3	.....
11.....	3	6	.....	23.....	2	1	.....
12.....	1	1	2	25.....	2	.....	.....
14.....	1	1	.....	Total.....	67	60	3
15.....	5	3	.....				
16.....	1	3	.....				

Table No. 7 refers to the same series of mosquitoes divided into groups of *A. punctipennis*, and *A. quadrimaculatus*, showing the number of bites obtained by each.

TABLE NO. 7.—*Giving the average number of bites on malarial carrier by mosquitoes arranged in groups.*

ANOPHELES PUNCTIPENNIS.

Cage number.	Number of mosquitoes applied.	Total number of bites.	Average number of bites per mosquito.
24.....	11	14	1.2
25.....	9	34	3.7
26.....	8	14	1.7
27.....	14	28	2.0
28.....	9	36	4.0
29.....	12	23	1.9
30.....	3	7	2.3
31.....	1	2	2.0

ANOPHELES QUADRIMACULATUS.

32.....	8	23	2.8
33.....	10	23	2.3
34.....	11	12	1.0
36.....	12	19	1.5
37.....	9	9	1.0
38.....	10	18	1.8

SUMMARY.

A. punctipennis.		A. quadrimaculatus.	
Number of mosquitoes.	Number of bites.	Number of mosquitoes.	Number of bites.
31.....	1-2	20.....	
15.....	2	22.....	
3.....	2-3	18.....	
9.....	3-4		
9.....	4		
671.....	* 2.3	601.....	* 1.7

<sup>1</sup> Total.

<sup>2</sup> Average.

The *Anopheles punctipennis* of this series were tested also for infectivity by feeding them upon a healthy volunteer. During a period of 34 days this individual was bitten 22 times, with negative results.

A total of 8 specimens of the 60 *Anopheles quadrimaculatus* employed in series 2 showed infection, as did also 1 of the 3 *Anopheles crucians*. The *A. quadrimaculatus* controls, which became infected, were dissected as follows: Three specimens, 11 days after biting the infected host, one 1 time and two 2 times; one specimen, 12 days after biting, twice; one specimen, 13 days after biting, 3 times; and

three specimens, 22 days after biting, one 1 time, one 2 times, and one 3 times. Gland sporozoites were seen in only one after 13 days and one after 22 days' incubation.

The oocysts in the infected *Anopheles crucians*, which had fed twice on a gamete carrier, had developed during 15 to 18 days prior to the dissection. Examination of this insect showed 8 well-defined mature oocysts on the wall of the mid-gut, 6 of these appearing to be ready for rupture, with liberation of their contained sporozoites.

#### SUMMARY.

1. Two hundred and nineteen specimens of *Anopheles punctipennis* Say were dissected from 3 to 38 days after multiple bites on individuals whose blood contained varying numbers of subtertian gametocytes (estivo-autumnal crescents). No infection was observed in the dissection of stomachs and salivary glands.

2. Two healthy individuals were bitten 91 and 180 times by specimens of *Anopheles punctipennis*, 4 to 33 days after sucking blood of a subtertian malarial carrier. In this experiment, and, subsequently in the employment of a healthy volunteer to feed 22 additional mosquitoes of this same species, *Anopheles punctipennis* Say could not be incriminated in the transmission of subtertian malarial fever.

The negative results in this experiment check only with the negative findings in the dissections of *A. punctipennis*, as it is recognized that the volunteers were not under absolute control; that is, because of the possible exposure to bites from infected anophelines while living in New Orleans.

3. Control feedings with 74 specimens of *Anopheles quadrimaculatus* Say resulted in an infection of 13.8 per cent, and with 3 specimens of *Anopheles crucians* Wied. of 33.3 per cent.

4. The coincidence in which one person developed subtertian malaria 11 days following the single bite of an *A. quadrimaculatus* that had become infected (as shown by dissection) 17 days previously by biting a heavily infected carrier, pointed strongly to this as the source of infection.

This might be offered as an additional check in the experiment, recognizing, however, the limitations that might be placed on it because of lack of absolute control of the volunteer living in New Orleans.